ATS LABS

AMENDMENT TO GLP TEST PROTOCOL

Amendment No.:	1
Effective Date:	January 26, 2009
Sponsor:	Summit Brands 7201 Engle Road Fort Wayne, IN 46804-2228
Sponsor Representative:	Wagner Regulatory Associates P.O. Box 640 Hockessin, DE 19707-0640
Test Facility:	ATS Labs 1285 Corporate Center Drive, Suite 110 Eagan, MN 55121
Protocol Title:	AOAC Use-Dilution Method
ATS Labs Protocol Number:	WRA01010609.UD
ATS Labs Project Number:	A07216

Modifications to Protocol:

This protocol is amended as follows:

1a. Due to the excessive foaming of the test substance during preparation, this protocol is amended to change the second sentence of the product preparation instructions on page 8 of the protocol from mixing the solution in a large flask using sterile bar for 3-4 minutes as follows:

Mix the solution in a sterile vessel by hand for 3-4 minutes.

1b. Per Sponsor's request, this protocol is amended to allow for repeat testing against *Staphylococcus aureus* to evaluate for potential false positives.

Changes to the protocol are acceptable as noted.

Study Director

1-26-09
Date

EXACT COPY
INITIALS JM DATE 2.10.09

(For Laboratory Use Only)
ATS Labs Project # A 07216
En 1/9/09

ATS LABS

PROTOCOL

AOAC Use-Dilution Method

Test Organisms:

Staphylococcus aureus (ATCC 6538)
Salmonella enterica (ATCC 10708)

PROTOCOL NUMBER

WRA01010609.UD

PREPARED FOR

Summit Brands
7201 Engle Road
Fort Wayne, IN 46804-2228

SPONSOR REPRESENTATIVE

Wagner Regulatory Associates
P.O. Box 640
Hockessin, DE 19707-0640

PERFORMING LABORATORY

ATS Labs
1285 Corporate Center Drive, Suite 110
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PREPARED BY

Jill Ruhme, B.S.
Research Scientist I

EXACT COPY
INITIALS JR DATE 2/10/09

DATE

January 6, 2009

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AOAC Use-Dilution Method

SPONSOR: Summit Brands
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Fort Wayne, IN 46804-2228

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TEST FACILITY: ATS Labs
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

PURPOSE
The purpose of this study is to determine the efficacy of the sponsor's product following the AOAC Use Dilution Method in compliance with the U.S. Environmental Protection Agency requirements set forth in the Pesticide Assessment Guidelines.

SUBSTANCE CHARACTERIZATION
Test substance characterization as to content, stability, etc., (40 CFR, Part 160, Subpart F [160.105]) is the responsibility of the Sponsor. The test substance shall be characterized by the Sponsor prior to the experimental start date of this study. Pertinent information, which may affect the outcome of this study, shall be communicated in writing to the Study Director upon sample submission to ATS Labs.

SCHEDULING AND DISCLAIMER OF WARRANTY
Experimental start dates are generally scheduled on a first-come/first-serve basis once ATS Labs receives the Sponsor approved/completed protocol, signed fee schedule and corresponding test substance(s). Based on all required materials being received at this time, the proposed experimental start date is January 20, 2009. Verbal results may be given upon completion of the study with a written report to follow on the proposed completion date of February 9, 2009. To expedite scheduling, please be sure all required paperwork and test substance documentation is complete/accurate upon arrival at ATS Labs.

If a test must be repeated, or a portion of it, due to failure by ATS Labs to adhere to specified procedures, it will be repeated free of charge. If a test must be repeated, or a portion of it, due to failure of internal controls, it will be repeated free of charge. "Methods Development" fees shall be assessed, however, if the test substance and/or test system require modifications due to complexity and difficulty of testing.

If the Sponsor requests a repeat test, they will be charged for an additional test.

Neither the name of ATS Labs or any of its employees are to be used in advertising or other promotion without written consent from ATS Labs.

The Sponsor is responsible for any rejection of the final report by the United States FDA or EPA concerning report format, pagination, etc. To prevent rejection, Sponsor should carefully review the ATS Labs final report and notify ATS Labs of any perceived deficiencies in these areas before submission of the report to the regulatory agency. ATS Labs will make reasonable changes deemed necessary by the Sponsor, without altering the technical data.

JUSTIFICATION FOR SELECTION OF THE TEST SYSTEM
The U.S. Environmental Protection Agency requires that a specific bacterial claim for a test substance intended for use on hard surfaces be supported by appropriate scientific data demonstrating the efficacy of the test substance against the claimed bacteria. This is accomplished in the laboratory by treating the target bacteria with the disinfectant (test substance) under conditions which simulate as closely as possible the actual conditions under which the test substance is designed to be used. For disinfectant products intended for use on hard surfaces (dry, inanimate environmental surfaces), a carrier method is used in the generation of the supporting data. The experimental design in this protocol meets these requirements.

TEST PRINCIPLE

A film of bacterial cells dried on a surface of stainless steel carriers is exposed to the test substance for a specified contact time. After exposure, the carriers are transferred to vessels containing neutralizing subculture media and assayed for survivors. Appropriate viability, carrier population and neutralization controls are performed. The current version of Standard Operating Procedure CGT-4400 reflects the methods which shall be used in this study.

TEST METHOD

Test Organisms	ATCC #	Growth Medium	Incubation Parameters
Staphylococcus aureus	6538	Synthetic Broth	35-37°C, aerobic
Salmonella enterica	10708	Synthetic Broth	35-37°C, aerobic

The test organisms to be used in this study were obtained from the American Type Culture Collection (ATCC), Manassas, VA.

Carriers

Carriers will be screened according to AOAC Official Method of Analysis and any carrier positive for growth will be discarded. Only penicylinders showing no growth may be used. Stainless steel penicylinders will be pre-soaked overnight in 1.0N NaOH, washed in water until neutral and autoclaved in 0.1% asparagine.

Preparation of Test Organisms

From a stock slant, an initial tube of culture broth will be inoculated. This culture is termed the "initial broth suspension." From this initial broth suspension, a minimum of three daily transfers will be performed on consecutive days prior to use in testing procedure. For each test organism, the appropriate growth medium will be subcultured using a daily transfer (more than 3, but less than 30 transfers) of the test organism.

A 48-54 hour broth culture incubated at the parameters listed above will be prepared.

The test cultures will be thoroughly mixed and allowed to stand for ≥10 minutes prior to use.

An organic soil load may be added to the test culture per Sponsor's request.

Contamination of Carriers

The penicylinders will be transferred to the culture and immersed for 15 minutes in a prepared suspension at a ratio of 1 carrier per 1.0 mL culture. The inoculated carriers will be dried on filter paper in a sterile petri dish at 35-37°C for 40 minutes. The drying conditions (temperature and humidity) will be appropriate for the test organism. The actual drying conditions will be clearly documented.

Preparation of Test Substance

The test substance(s) to be assayed will be used as directed by the Sponsor. If a dilution of the test substance is requested by the Sponsor, the diluted test substance(s) shall be used within three hours of preparation. Ten (10) mL of the test substance at its use-dilution will be aliquoted into the required number of sterile 25 x 150 mm tubes. The tubes will be placed into a waterbath at the specified exposure temperature, and allow to equilibrate for ≥10 minutes prior to testing.

Exposure Conditions

Each contaminated and dried carrier will be placed into a separate tube containing 10 mL of the test substance at its use-dilution for the desired exposure time and temperature.

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Test System Recovery
Following the Sponsor specified exposure period, each medicated carrier will be transferred by wire hook at staggered intervals to 10 mL of neutralizing broth. If necessary, carriers will be transferred into individual secondary subculture tubes containing 10 mL neutralizing broth ≥30 minutes after subculture of first carrier.

Incubation and Observation
All subculture tubes and plates will be incubated for 48±4 hours at 35-37°C (or other appropriate time/temperatures).

Following incubation, the subculture tubes will be visually examined for growth. If necessary, the subcultures may be placed at 2-8°C for up to three days prior to examination.

Representative subculture tubes demonstrating growth (positive tubes) will be subcultured onto appropriate agar for confirmation of the test organism.

STUDY CONTROLS

Purity Control
A "streak plate for isolation" will be performed on the organism culture and following incubation examined in order to confirm the presence of a pure culture. The acceptance criterion for this study control is a pure culture demonstrating colony morphology typical of the test organism.

Organic Soil Sterility Control
The serum used for soil load will be cultured, incubated, and visually examined for lack of growth. The acceptance criterion for this study control is lack of growth.

Carrier Sterility Control
A representative uninoculated carrier will be added to the neutralizing subculture medium. The subculture medium containing the carrier will be incubated and examined for growth. The acceptance criterion for this study control is lack of growth.

Neutralizing Subculture Medium Sterility Control
A representative sample of uninoculated neutralizing subculture medium will be incubated and visually examined. The acceptance criterion for this study control is lack of growth.

Viability Control
A representative inoculated carrier will be added to the subculture medium. The subculture medium containing the carrier will be incubated and visually examined for growth. The acceptance criterion for this study control is growth.

Neutralization Confirmation Control
The neutralization of the test substance will be confirmed by exposing sterile carriers (representing not less than 10% of the total number of test carriers) to the test substance and transferring them to primary subcultures containing 10 mL of neutralizing subculture medium. If performed in the test procedure, carriers will then be transferred from primary subcultures into individual secondary subcultures ≥30 minutes following the primary transfer. The subcultures containing the exposed carriers will be inoculated with ≤100 colony forming units (CFU) of each test organism, incubated under test conditions and visually examined for the presence of growth. This control will be performed with multiple replicates using different dilutions of the test organism. A standardized spread plate procedure will be run concurrently in order to enumerate the number of CFU actually added. The control result will be reported using data from the most appropriate dilution.

The acceptance criterion for this study control is growth following inoculation with ≤100 CFU.

OR:

Ten percent of the subcultures containing carriers showing no growth will be inoculated with ≤100 CFU of each test organism and incubated. This control will be performed with multiple replicates representing different dilutions of the test organism. A standardized spread plate procedure will be run concurrently in order to enumerate the number of CFU actually added. The control result will be reported using data from the most appropriate dilution.

The acceptance criterion for this study control is growth following inoculation with ≤100 CFU.

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Carrier Population Control
Inoculated carriers will be added at a ratio of 1 carrier to 10 mL neutralizing broth and vortex mixed. Appropriate serial ten-fold dilutions will be prepared and the aliquots spread plated on agar plate medium, and incubated. Following incubation, the resulting colonies will be enumerated and the CFU/carrier calculated. The acceptance criterion for this study control is a minimum of 1.0×10^4 CFU/carrier.

PROCEDURE FOR IDENTIFICATION OF THE TEST SYSTEM
ATS Labs maintains Standard Operating Procedures (SOPs) relative to efficacy testing studies. Efficacy testing is performed in strict adherence to these SOPs which have been constructed to cover all aspects of the work including, but not limited to, receipt, log-in, and tracking of biological reagents including bacterial strains for purposes of identification, receipt and use of chemical reagents. These procedures are designed to document each step of efficacy testing studies. Appropriate references to medium batch number, etc. are documented in the raw data collected during the course of each study.

Additionally, each efficacy test is assigned a unique Project Number when the protocol for the study is initiated by the Study Director. This number is used for identification of the test subculture tubes, etc. during the course of the test. Test subculture tubes are also labeled with reference to the test organism, experimental start date, and test product. Microscopic and/or macroscopic evaluations of positive subcultures are performed in order to confirm the identity of the test organism. These measures are designed to document the identity of the test system.

METHOD FOR CONTROL OF BIAS: NA

STUDY ACCEPTANCE CRITERIA

Test Substance Performance Criteria
The EPA efficacy performance requirements for label claims state that the disinfectant must kill the microorganism on 59 out of the 60 inoculated carriers.

Control Acceptance Criteria
The study controls must perform according to the criteria detailed in the study controls description section.

REPORT
The report will include, but not be limited to, identification of the sample, date received, initiation and completion dates, identification of the bacterial strains used, description of media and reagents, description of the methods employed, tabulated results and conclusion as it relates to the purpose of the test, and all other items required by 40 CFR Part 160.185.

PROTOCOL CHANGES
If it becomes necessary to make changes in the approved protocol, the revision and reasons for changes will be documented, reported to the Sponsor and will become a part of the permanent file for that study. Similarly, the Sponsor will be notified as soon as possible whenever an event occurs that may have an effect on the validity of the study.

Standard operating procedures used in this study will be the correct effective revision at the time of the work. Any minor changes to SOPs (for this study) or methods used will be documented in the raw data and approved by the Study Director.

PRODUCT DISPOSITION
It is the responsibility of the Sponsor to retain a sample of the test substance. All unused test substance will be discarded following study completion unless otherwise indicated by Sponsor.

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RECORD RETENTION

Study Specific Documents

All of the original raw data developed exclusively for this study shall be archived at ATS Labs. These original data include, but are not limited to, the following:

1. All handwritten raw data for control and test substances including, but not limited to notebooks, data forms and calculations.
2. Any protocol amendments/deviation notifications.
3. All measured data used in formulating the final report.
4. Memoranda, specifications, and other study specific correspondence relating to interpretation and evaluation of data, other than those documents contained in the final study report.
5. Original signed protocol.
6. Certified copy of final study report.
7. Study-specific SOP deviations made during the study.

Facility Specific Documents

The following records shall also be archived at ATS Labs. These documents include, but are not limited to, the following:

1. SOPs which pertain to the study conducted.
2. Non study-specific SOP deviations made during the course of this study which may affect the results obtained during this study.
3. Methods which were used or referenced in the study conducted.
4. QA reports for each QA inspection with comments.
5. Facility Records: Temperature Logs (ambient, incubator, etc.), Instrument Logs, Calibration and Maintenance Records.
6. Current curriculum vitae, training records, and job descriptions for all personnel involved in the study.

REFERENCES

1. Association of Official Analytical Chemists (AOAC), 1990. Use-Dilution Tests, p. 135-137. *In* Official Methods of Analysis of the AOAC, Fifteenth Edition.
2. Association of Official Analytical Chemists (AOAC), 1990. Germicidal and Detergent Sanitizing Action of Disinfectants, p. 139 [Preparation of Synthetic Hard Water]. *In* Official Methods of Analysis of the AOAC, Fifteenth Edition.
3. U.S. Environmental Protection Agency, Registration Division, Office of Pesticide Programs, 1982. Efficacy Data Requirements, Disinfectants for Use on Hard Surfaces, DIS/TSS-1.
4. U.S. Environmental Protection Agency, Registration Division, Office of Pesticide Programs, 1979. Efficacy Data Requirements, Supplemental Recommendations, DIS/TSS-2.
5. U.S. Environmental Protection Agency, Registration Division, Office of Pesticide Programs, 1982. Subseries 91A: Public Health Uses. *In* Pesticide Assessment Guidelines – Subdivision G (Product Performance).

DATA ANALYSIS

Calculations

Carrier Population Control Calculation:

$$\text{Carrier population, CFU/carrier} = \frac{\text{(average number colonies/plate @ dilution) x (dilution factor) x (volume neutralizer)}}{\text{(number of carriers tested) x (volume plated)}}$$

The carrier population is calculated and reported using data from the most appropriate dilution(s).

Statistical Analysis

None used.

STUDY INFORMATION

(All sections must be completed prior to submitting protocol)

Sponsor (Date/Initial): 1/7/2009 JMM

Test Substance (Name & Batch Numbers, *including ≥60 day old batch* - exactly as it should appear on final report):
Summit Brands Disposer Care Garbage Disposer Cleaner Sanitizer Disinfectant, Batch 08

Specify ≥60 day old batch: NA

Expiration Date: NA

Product Description:
- ☐ Quaternary ammonia
- ☐ Iodophor
- ☐ Sodium hypochlorite
- ☐ Peracetic acid
- ☐ Peroxide
- ☑ Other sodium dichloro s-triazinetrione dihydrate

Test Substance Active Concentration (upon submission to ATS Labs): 2.0%

Neutralization/Subculture Broth: ☒ USE SAME AS PROJECT NO. A06341
ATS Labs' Discretion. By checking, the Sponsor authorizes ATS Labs, at their discretion, to perform neutralization confirmation assays at the Sponsor's expense prior to testing to determine the most appropriate neutralizer. (See Fee Schedule).

Storage Conditions:
- ☑ Room Temperature
- ☐ 2-8°C
- ☐ Other:

Letheen Broth with 0.07% Lecithin + 0.5% Tween 80 + 0.1% Sodium Thiosulfate
JM 1-8-09

Hazards:
- ☑ None known: Use Standard Precautions
- ☐ Material Safety Data Sheet, Attached for each product
- ☐ As Follows:

Product Preparation
- ☐ No dilution required, Use as received (RTU)
- ☑ *Dilutions/Concentrations to be tested See instructions below
  - ☐ Deionized Water (Filter Sterilized)
  - ☐ Tap Water (Filter Sterilized)
  - ☑ AOAC Synthetic Hard Water: 400 PPM
  - ☐ Other

Note: An equivalent dilution may be made unless otherwise requested by the Sponsor.

Test Organism(s): *Staphylococcus aureus* (ATCC 6538)
Salmonella enterica (ATCC 10708)

Carrier Number: 60 per batch

Exposure Time: 10 Minutes

Exposure Temperature: 20 ± 1 °C

Organic Soil Load:
- ☑ Minimum 5% Organic Soil Load (Fetal Bovine Serum)
- ☐ No Organic Soil Load Required
- ☐ Other:

USE DILUTION INSTRUCTIONS - Dissolve the contents of one packet in 16 fluid ounces of AOAC Synthetic hard water heated to 48-51°C. Mix the solution in a large flask using a sterile bar for 3-4 minutes. Cut up the packet into ½ by ½ inch squares and add to the flask. Continue to stir briefly to thoroughly incorporate the the packet and any remaining powder. Allow the foaming to subside for 3-4 minutes. Prepared product should be made immediately prior to medication of test carriers. Entire preparation of the test substance should take approximately 10 minutes.

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TEST SUBSTANCE SHIPMENT STATUS

☐ Has been used in one or more previous studies at ATS Labs .
☑ Has been shipped to ATS Labs (but has not been used in a previous study).
Date shipped to ATS Labs: 11/13/08 Sent via *overnight* delivery? ☐ Yes ☐ No
☐ Will be shipped to ATS Labs.
Date of expected receipt at ATS Labs: _____
☐ Sender (if other than Sponsor): _____

COMPLIANCE

Study to be performed under EPA Good Laboratory Practice regulations (40 CFR Part 160) and in accordance to standard operating procedures.
☑ Yes
☐ No (Non-GLP Study)

PROTOCOL MODIFICATIONS

☒ Approved without modification
☐ Approved with modification - Supplemental Information Form Attached - ☐ Yes ☐ No

APPROVAL SIGNATURES

SPONSOR:

NAME: Jim Wagner (Summit Brands) TITLE: Agent for Summit Brands

SIGNATURE: James Wagner DATE: 1/7/2009

PHONE: 302-234-8550 FAX: 302-234-7570 EMAIL: james@wagnerreg.com

For confidentiality purposes, study information will be released only to the sponsor/representative signing the protocol (above) unless other individuals are specifically authorized in writing to receive study information.

Other individuals authorized to receive information regarding this study: ☐ See Attached

ATS Labs:

NAME: Jill Ruhme
Study Director

SIGNATURE: Jill Ruhme DATE: 1-8-09
Study Director

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